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Evaluation of the A/Seal/Mass/1/80 Virus in Squirrel Monkeys

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An influenza A virus isolated from seals [A/Seal/Mass/1/80 (H7N7)] and an isolate of this virus obtained from a human conjunctiva were evaluated for replication and virulence in squirrel monkeys. When the seal virus was administered intratracheally, it replicated in lungs and nasopharynges and induced illness almost to the same extent that a human influenza A virus [A/Udorn/72 (H3N2)] did. In one monkey that died of pneumonia, the seal virus was recovered from spleen, liver, and muscle as well as lung. After conjunctival administration in monkeys, the seal virus replicated to a peak titer in the conjunctivae 30-fold greater than that attained by the human virus, but this difference was not statistically significant. In contrast, the seal virus replicated less well than the human virus in the tracheae and nasopharynges when administered by the conjunctival route. These results indicate that the seal virus can replicate efficiently in primates, that it can spread systemically, and that it might differ from human virus in being able to replicate slightly better in primate conjunctival tissue.

Recently, an influenza A virus that was genetically and antigenically related to avian influenza A viruses was isolated from harbor seals (2, 4, 8). This virus, designated A/Seal/Mass/1/80 (H7N7), was isolated from the lungs and brains of seals dying of acute pneumonia. The virus was also isolated from the conjunctiva of a man in contact with an infected seal (7).

Squirrel monkeys have been used to assess the virulence of a variety of human and avian influenza A viruses (5, 6). The human viruses replicated to high titer in the nasopharynges and tracheae of squirrel monkeys and induced local rhinitis with some systemic symptoms (5, 6). The avian influenza A viruses manifested a spectrum of virulence, ranging from some strains that were avirulent but infectious to others that replicated to almost the same extent and that caused the same level of illness as a human influenza A virus (5). In the present study, we sought to examine the virulence of the seal virus for squirrel monkeys by both intratracheal and conjunctival routes of virus administration. The conjunctival route of administration was chosen to assess the level of replication of the seal virus in primate conjunctivae and to compare it with that of a human virus, the A/Udorn/72 (H3N2) strain, which is known to be virulent for squirrel monkeys (5).

The A/Seal/Mass/1/80 virus (H7N7), which was isolated from the lung of a seal, was an uncloned suspension of virus in its third egg passage that had a titer of $10^{7.3}$ 50% tissue culture infective doses ($TCID_{50}$) per ml. The A/Seal/Mass/1/80 virus that was isolated from the human eye (uncloned) was also in its third egg passage and had a titer of $10^{7.3}$ $TCID_{50}$/ml. The influenza A/Udorn/307/72 (H3N2) virus ($10^{8.0}$ $TCID_{50}$/ml) was a cloned virus known to be virulent for squirrel monkeys (5). The methods for intratracheal administration of virus and for the housing, sampling, and observation of the squirrel monkeys have been previously described, as have the methods for titrating nasopharyngeal swabs and tracheal lavage fluids (5). To administer virus by the conjunctival route, 0.5-ml inocula containing $10^{7.0}$ $TCID_{50}$ of virus each were placed in the lower eyelids of monkeys anesthetized with ketamine. The A/Seal/Mass/1/80 whole virus was used in the hemagglutination inhibition (HI) test as described (8).

The level of replication of the two seal influenza A viruses after intratracheal administration in the monkeys is presented and compared with that of the human virus in Table 1. The data for the human virus were previously published and are included here for comparison (5). The seal virus replicated in nasopharynges and tracheae for the same duration and to similar peak titers as the human influenza A virus. The average

TABLE 1. Evaluation of seal influenza virus in squirrel monkeys: intratracheal administration of virus[a]

Virus	No. of monkeys tested	Viral replication				Serum HI antibody response[b]		Clinical response (days with illness ± SE)[c]
		Nasopharynx		Tracheal lavage				
		Duration of virus shedding (days ± SE)[d]	Mean peak titer (log_{10} $TCID_{50}$/ml ± SE)[e]	Duration of virus shedding (days ± SE)[f]	Mean peak titer (log_{10} $TCID_{50}$/ml ± SE)[e]	Reciprocal mean log_2 titer ± SE (day 28)	Monkeys with fourfold or greater rise	
A/Seal/Mass/1/80 (seal lung isolate)	4	5.8 ± 1.7	4.1 ± 0.4	4.5 ± 0.5	5.5 ± 0.5	5.3 ± 0.5	4	6.0 ± 2.0
A/Seal/Mass/1/80 (human eye isolate)	4	7.3 ± 1.0	5.9 ± 0.4	4.5 ± 0.5	5.6 ± 0.1	4.3 ± 0.3[g]	3[g]	7.4 ± 0.3
A/Udorn/307/72 (human virus)	11	5.6 ± 0.7	5.0 ± 0.4	5.3 ± 0.4	6.3 ± 0.2	6.8 ± 0.4	11	7.1 ± 1.0

[a] Each monkey received $10^{7.0}$ $TCID_{50}$ of virus intratracheally in an 0.5-ml inoculum and was infected as determined by recovery of virus.

[b] Reciprocal serum HI antibody titer (log_2) before inoculation was ≤ 1.0 for each monkey.

[c] For eight control animals, the average number of days with illness was 0.3 ± 0.3.

[d] Each monkey was tested daily for 10 days.

[e] Amount of virus in the nasopharyngeal swab or tracheal lavage from each monkey was determined, and the maximum amount shed by each monkey was averaged.

[f] Each monkey was tested on days 2, 4, and 6.

[g] One monkey died on day 10, and only three monkeys were tested.

numbers of days of illness for the seal and human viruses were also comparable. The illness experienced by the monkeys infected with the seal virus consisted of rhinitis, systemic symptoms, or both. The systemic symptoms consisted of lethargy and anorexia. One monkey infected with the human eye isolate died of pneumonia on the 10th day after virus administration. Virus was isolated from suspensions (10%, wt/vol) of lung ($10^{5.75}$ $TCID_{50}$/ml), trachea ($10^{4.75}$ $TCID_{50}$/ml), spleen ($10^{3.0}$ $TCID_{50}$/ml), liver ($10^{3.75}$ $TCID_{50}$/ml), and muscle ($10^{1.75}$ $TCID_{50}$/ml). Virus was not recovered from the brain or kidneys of the dead animal. Blood was not taken from the dead monkey for analysis. Two control placebo-inoculated animals that were similarly handled and sampled did not manifest local or systemic illness.

Four monkeys received the seal virus (human eye isolate) by the conjunctival route, and the virus shedding from the nasopharynges, tracheae, and conjunctivae was determined (Table 2). Similarly, four monkeys were infected by the conjunctival route with the A/Udorn/72 human virus. The seal and human virus replicated to the same peak titer in the nasopharynges, although the duration of shedding of the human virus was longer. However, the human virus replicated for a longer duration and to higher titers in tracheae. In contrast, the seal virus initiated a more reproducible infection in the conjunctivae, with the peak titer of virus achieved being 30-fold greater than those achieved by the human virus. However, the difference was not statistically significant given the small number of animals studied. Conjunctivitis was not observed in any of the animals, but two animals inoculated with the seal virus developed mild rhinitis.

The present results indicate that the seal influenza A virus can replicate in the nasopharynges and tracheae of squirrel monkeys to titers comparable with those of a virulent human virus. In addition, illness was also induced for a comparable duration. The seal virus resembles in this regard two avian influenza A viruses, A/Mallard/N.Y./6874/78(H3N2) and A/Pintail/Alb/121/79(H7N8), which also replicated to high titers in the upper and lower respiratory tracts of squirrel monkeys (5).

In seals, A/Seal/Mass/1/80 virus spread systemically and could be recovered from the brains of a majority of the animals sampled (8). In squirrel monkeys, evidence for systemic spread of this virus was also obtained with spleen, liver, and muscle tissue yielding virus, whereas virus was not obtained from the brain or kidneys of the same monkey. Some avian viruses are known to cause systemic infections in their natural hosts, whereas human viruses are only rarely isolated from sites other than the respiratory tract (3). This systemic spread might be an isolated phenomenon occurring in an animal with an overwhelming pulmonary infection. Alternatively, the A/Seal/Mass/80 virus might have a much greater tendency to cause a systemic infection, and in this regard it resem-

TABLE 2. Evaluation of seal influenza virus (human eye isolate) in squirrel monkeys: conjunctival administration of virus ($10^{7.0}$ $TCID_{50}$)

Virus	Monkey no.	Viral replication						Serum HI antibody (reciprocal log_2 titer on day 28)[a]	Illness
		Nasopharynx		Tracheal lavage		Conjunctiva[b]			
		Days of virus shedding[c]	Peak titer of virus (log_{10} $TCID_{50}$/ml)	Days of virus shedding[d]	Peak titer of virus (log_{10} $TCID_{50}$/ml)	Days of virus shedding[c]	Peak titer of virus (log_{10} $TCID_{50}$/ml)		
A/Seal/Mass/1/80	470	3	4.5	0	<0.5	2	4.0	4	None
	471	2	4.5	2	3.0	2	5.0	3	None
	480	4	5.0	0	<0.5	1	2.5	2	Rhinitis (3 days)
	481	4	5.0	2	1.0	4	3.0	2	Rhinitis (4 days)
A/Udorn/307/72	472	5	5.0	0	<0.5	1	1.5	7	None
	473	7	4.5	6	6.0	1	1.5	7	None
	542	6	3.5	6	6.5	2	2.5	8	Rhinitis (3 days)
	341	7	6.5	6	5.0	7	4.0	8	None

[a] Reciprocal serum HI antibody titer (log_2) before inoculation was ≤ 1.0 in each monkey.
[b] The conjunctiva was sampled for virus shedding by flushing 1.0 ml of phosphate-buffered saline across the cornea and conjunctiva of each eye and pooling the washes.
[c] Each monkey was tested daily for 10 days.
[d] Each monkey tested on days 2, 4, and 6.

bles certain avian influenza A virus infections of birds.

Purulent conjunctivitis is an infrequent manifestation of influenza A virus infection in humans, but conjunctival inflammation is relatively common (1). Thus, it was surprising to observe conjunctivitis in individuals in contact with seals or seal tissues infected with the seal influenza virus. The seal virus may replicate more efficiently in primate conjunctival tissue than human viruses, but this could not be statistically confirmed with the limited number of animals studied.

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LITERATURE CITED

1. **Douglas, R. G., Jr.** 1975. Influenza in man, p. 395–447. *In* E. D. Kilbourne (ed.), The influenza viruses and influenza. Academic Press, Inc., New York.
2. **Geraci, J. R., D. J. St. Aubin, I. K. Barker, R. G. Webster, V. S. Hinshaw, W. J. Bean, H. L. Ruhnke, J. H. Prescott, G. Early, A. S. Baker, S. Madoff, and R. T. Schooley.** 1982. Mass mortality of harbor seals: pneumonia associated with influenza A virus. Science **215**:1129–1131.
3. **Hinshaw, V. S., and R. G. Webster.** 1982. The natural history of influenza A viruses, p. 79–104. *In* A. S. Beare (ed.) Basic and applied influenza research. CRC Press, Boca Raton, Fla.
4. **Lang, G., A. Gagnon, and J. R. Geraci.** 1981. Isolation of an influenza A virus from seals. Arch. Virol. **68**:189–195.
5. **Murphy, B. R., V. S. Hinshaw, D. L. Sly, W. T. London, N. T. Hosier, F. T. Wood, R. G. Webster, and R. M. Chanock.** 1982. Virulence of avian influenza A viruses for squirrel monkeys. Infect. Immun. **37**:1119–1126.
6. **Murphy, B. R., D. L. Sly, N. T. Hosier, W. T. London, and R. M. Chanock.** 1980. Evaluation of three strains of influenza A virus in humans and in owl, cebus, and squirrel monkeys. Infect. Immun. **28**:688–691.
7. **Webster, R. G., J. R. Geraci, G. Petursson, and K. Skirnisson.** 1981. Conjunctivitis in human beings caused by influenza A virus of seals. N. Engl. J. Med. **304**:911.
8. **Webster, R. G., V. S. Hinshaw, W. J. Bean, K. L. van Wyke, J. R. Geraci, D. J. St. Aubin, and G. Petursson.** 1981. Characterization of an influenza A virus from seals. Virology **113**:712–724.